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Effect of depleted uranium on a soil microcosm fungal community and influence of a plant-ectomycorrhizal association

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ABSTRACT

Fungi are one of the most biogeochemically active components of the soil microbiome, becoming particularly important in metal polluted terrestrial environments. There is scant information on the mycobiota of uranium (U) polluted sites and the effect of metallic depleted uranium (DU) stress on fungal communities in soil has not been reported. The present study aimed to establish the effect of DU contamination on a fungal community in soil using a culture-independent approach, fungal ribosomal intergenic spacer analysis (F-RISA). Experimental soil microcosms also included variants with plants (*Pinus silvestris*) and *P. silvestris/Rhizopogon rubescens* ectomycorrhizal associations. Soil contamination with DU resulted in the appearance of RISA bands of the ITS fragments of fungal metagenomic DNA that were characteristic of the genus *Mortierella* (Mortierellomycotina: Mucoromycota) in pine-free microcosms and for ectomycorrhizal fungi of the genus *Scleroderma* (Basidiomycota) in microcosms with mycorrhizal pines. The precise taxonomic affinity of the ITS fragments from the band appearing for non-mycorrhizal pines combined with DU remained uncertain, the most likely being related to the subphylum Zoopagomycotina. Thus, soil contamination by thermodynamically unstable metallic depleted uranium can cause a significant change in a soil fungal community under experimental conditions. These changes were also strongly affected by the presence of pine seedlings and their mycorrhizal status which impacted on DU biocorrosion and the release of bioavailable uranium species.

Keywords: Depleted uranium, F-RISA, Fungal communities, Mycorrhiza, Soil

Introduction

Fungi are a fundamental component of the soil microbiome with a major role in many important metal, radionuclide and mineral transformations, although often neglected within geomicrobiology compared to prokaryotic microorganisms (Gadd 2007, 2008, 2010; Gadd and Raven 2010; Sullivan and Gadd 2019). Fungal properties are dependent on their filamentous branching growth habit and chemoorganotrophic metabolism and these enable effective colonization and alteration of organic and inorganic substrates, the latter leading to changes in metal mobility and the stability of metal-containing minerals (Boswell *et al*. 2003; Gadd 2007; Gadd *et al*. 2005; Lloyd and Gadd 2011; Vázquez-Campos *et al*. 2015; Rangel *et al*. 2018; Ceci *et al*. 2019; Song *et al*. 2019). Fungal interactions with uranium chemical species have been little studied compared to bacterial subsurface uranium transformations (Gadd and Fomina 2011; Lloyd and Gadd 2011). Such interactions are not only relevant to understanding the geomicrobiology of uranium, but also to the bioremediation of contaminated terrestrial habitats. Considering the dependence of almost all land plants on symbiotic mycorrhizal fungi and the fact that many mycorrhizal fungal species are capable of uranium transformations, these biogeochemical activities may be of importance in phyto- and/or other bioremediation strategies for soils polluted with various forms of uranium.

Uranium is a complex element capable of forming a variety of different chemical species in the environment (Van Haverbeke *et al*. 1996; Suzuki and Banfield 1999; Gorman-Lewis *et al*. 2008). One of the least studied forms of uranium is ammunition metallic depleted uranium (DU; a 97.25% U: 0.75% Ti alloy) which has been actively tested and used during war campaigns over the last three decades (Mellini and Riccobono 2005; Salbu *et al*. 2005). Metallic depleted uranium is thermodynamically unstable and undergoes oxidation and corrosion which results in leaching of mobile uranium species into the environment (Mellini and Riccobono 2005). Previously, it has been demonstrated that many soil saprotrophic and mycorrhizal fungi exhibit a

high uranium tolerance and the ability to solubilize uranium oxides and metallic depleted uranium with subsequent re-precipitation of secondary uranyl phosphate minerals that are capable of long-term uranium retention (Fomina *et al*. 2007; 2008). These minerals belong to the meta-autunite group (uramphite and/or chernikovite) and, in the case of depleted uranium, DU-encrusted fungal hyphae could accumulate as much as 300–400 mg U g dry wt^{-1} (Fomina *et al*. 2008). It has also been shown that in the presence of uranium nitrate, common saprotrophic fungi and yeasts can also form a range of uranyl phosphate minerals (Liang *et al*. 2015, 2016).

There is scant information on the mycobiota of uranium polluted sites. In studies on the microbiota of uranium-polluted soil at the Ranger Uranium Mine (Northern Territory, Australia), it was found that saprotrophic fungi outcompeted bacteria at increased uranium concentrations under the selected experimental conditions (Mumtaz *et al*. 2013). The dominance of members of the Ascomycota and a pronounced shift in fungal community composition across a groundwater pH gradient with reduced species diversity at pH < 4.5 were found in a subsurface environment co-contaminated with uranium and nitrate (Jasrotia *et al*. 2014). It was also found that strains of *Tricladium splendens* Ingold and *Varicosporium elodeae* Kegel isolated from an abandoned uranium mine drainage stream showed higher adaptive capabilities to uranium than isolates from a reference control stream (Ferreira *et al*. 2010). Analysis of ectomycorrhizal (EM) community structure in uranium mining heaps (near Ronneburg, Thuringia, Germany) revealed that all EM fungal species dominating in the highly uranium-contaminated sites were also present at the least polluted reference site (Staudenrausch *et al*. 2005). However, the potential effects of DU on soil fungal communities have never been reported. The aim of the current study was to evaluate the effect of DU contamination on the soil fungal community in experimental microcosms, with or without plants using a culture-independent F-RISA-based approach.

Methods and materials

Ectomycorrhizal fungal strain and growth conditions

The mycobiont used for ectomycorrhiza synthesis was the ectomycorrhizal fungus *Rhizopogon rubescens* Tulasne (Basidiomycota, Order Agaricomycetes, Class Boletales: common name Blushing False Truffle) strain B32o, isolated from a root tip in unpolluted alkaline soil, Öland Island, Sweden (kindly provided by Prof. H. Wallander). *R. rubescens* B32o has previously been shown to be biogeochemically active exhibiting a high tolerance to uranium oxides and able to transform uranium oxides and metallic depleted uranium into secondary uranium-bearing minerals (Fomina *et al*. 2007, 2008). *R. rubescens* B32o was maintained at 25°C on a modified Melin–Norkrans (MMN) agar medium comprising $(NH_4)_2HPO_4$ (500 mg l^{-1}), KH_2PO_4 (300 mg l^{-1}), $MgSO_4 \cdot 6H_2O$ (140 mg l^{-1}), $CaCl_2 \cdot 7H_2O$ (50 mg l^{-1}), NaCl (25 mg l^{-1}), D-glucose (10 g l^{-1}), glutamic acid (1 mg l^{-1}), thiamine (0.1 mg l^{-1}), and agar no. 1 (Lab M, Bury, UK) (14 g l^{-1}) in Milli-Q water. Before adding the agar and prior to autoclaving, the liquid medium was adjusted to pH 5.5 using concentrated HCl.

Synthesis of ectomycorrhiza

Synthesis of *R. rubescens*/Scots pine ectomycorrhiza was carried out under sterile conditions using a test-tube technique (Peterson and Chakravarty 1991). Seeds of *Pinus sylvestris* L. (Chiltern Seeds, Ulverston, Cumbria, UK) were surface-sterilized in 30% hydrogen peroxide with one drop of Tween 60 for 30 min, then thoroughly washed with sterile Milli-Q water. They were then transferred to Petri dishes containing MMN agar medium and the seeds left to germinate for at least 14 days. Synthesis of ectomycorrhizas was performed in autoclaved (120˚C, 60 min) glass tubes (culture tubes 25 × 150 mm, Sigma-Aldrich, Dorset, UK) filled with 30 ml vermiculite (Sinclair, Lincoln, UK) and peat (Irish Moss Peat, B & Q plc, Chandlers Ford, UK) mixture 5:1 and 10 ml of Ingestad's medium comprising K_2SO_4 (12.25 mg l^{-1}), KH_2PO_4 (8.58 mg l^{-1}), K_2HPO_4 (10.11 mg l^{-1}), KNO_3 (9.71 mg l^{-1}), NH_4NO_3 (58.56 mg l^{-1}), $Ca(NO_3)_2$ (8.61 mg l^{-1}), $Mg(NO_3)_2$ (15.84 mg l^{-1}), HNO_3 (13.7 mg l^{-1}), H_3BO_3 (0.57 mg l^{-1}), $Mn(NO_3)_2$

(0.913 mg l^{-1}), $Zn(NO_3)_2$ (0.07 mg l^{-1}), $CuCl_2$ (0.04 mg l^{-1}), Na_2MoO_4 (0.009 mg l^{-1}), and FeNaEDTA (1.149 mg l^{-1}) in Milli-Q water (Ingestad 1979). D-Glucose (0.5 g l^{-1}) was also included in the medium to initiate fungal growth in the matrix. Germinating pine seeds were planted into the vermiculite/peat matrix together with four 7-mm diameter disks of mycelium cut from the leading edge of colonies that had been maintained on MMN at 25˚C for at least 14 days. Seedlings were cultivated for 3–4 months in a growth chamber (Fisons Fitotron 600 Growth Cabinet, Suffolk, UK) with 200 mmol m^{-2} s^{-1} photosynthetically active radiation (PAR), at least 60% relative air humidity, and a day/night regime of 18/6 h and a temperature of 23/15°C. Mycorrhizal colonization of the roots was nearly 85–90%. After successful colonization, uniform pine seedlings were carefully removed from tubes, washed with sterile Milli-Q water, and transferred into jars (Peterson and Chakravarty 1991) to be used in microcosm experiments. The pine seeds for non-mycorrhizal seedlings to be used in further microcosm experiments were prepared as described above without infecting with the ectomycorrhizal fungus.

Depleted uranium

The samples of DU alloy (provided by Defence Science and Technology Laboratories, Porton Down, UK and machined by AWE Aldermaston, Reading, UK) were made in isosceles triangular shapes weighing approx. 6.5–8.5 g with approximate dimensions (mm): 15 × 15 × 11 and 5 mm in height, with specific radioactivity being approximately 12.5 kBq g^{-1} (Fig. 1). Before use, they were sequentially washed with dichloromethane and isopropyl alcohol and sterilized with absolute ethanol (Fomina *et al*. 2008). The DU samples were then covered with soil in the microcosms. After incubation in soil for 12 months, the weathered DU samples were removed from the matrix. The uranium content in different chemical fractions (water soluble fraction extracted for 30 min at 80°C; 1 M acetic acid fraction extracted for 2 days at room temperature; concentrated nitric acid extracted for 1 day at room temperature) in the microcosm soil was analysed by ICP-AES (Perkin-Elmer 5300 Optima dual view inductively-coupled plasma-atomic emission spectrometer) (Fomina *et al*. 2008). ICP-AES analyses of uranium accumulated in plants was performed on biomass digested in

concentrated HNO_3 as previously described (Fomina *et al*. 2007). SigmaStat (Release 3.1) was used for statistical analyses.

Soil microcosms

The microcosms were designed to simulate pollution of a soil ecosystem with metallic depleted uranium. Autoclaved 60 × 150 mm glass jars (350 ml Tesco "Olives" jars) were used as modified Mason jars in the microcosm experiments (Fomina *et al*. 2006) (Fig. 2). To improve drainage, 25 cm^3 of sterile acid-washed glass beads (diameter approx. 3mm) was placed at the bottom of the jars. 100 cm^3 of sieved (2 mm mesh size) non-sterile uncontaminated sandy soil collected from dune sand at Drigg Coast, Cumbria, UK was then placed on top of the beads. Holes (10 mm diameter) were punched in the jar caps and plugged with autoclaved foam to provide gas exchange for the pine seedlings. Caps were sealed to the jars using autoclaved lanolin (Sigma-Aldrich, Gillingham, Dorset, UK). Variants of microcosms included those with DU and uranium-free control (codes /U and /O) as well as mycorrhizal and non-mycorrhizal pine seedlings (codes M/ and N/), and, finally, soil without plants (O/) (Table 1). At least three replicates for each treatment were used. Pine seedlings were watered with 25 ml sterile Ingestad's medium after planting and incubated in a growth chamber (Fisons Fitotron 600 Growth Cabinet) with 200 mmol m^{-2} s^{-1} PAR, at least 60% relative air humidity, and a day/night regime of 18/6 h and a temperature of 23/15°C. The same treatment was applied to the plant-free variants. Every second month, pine seedlings were watered with 25 ml sterile water. The bottoms of the jars were covered with foil to keep the soil of the microcosms in the dark. The samples of depleted uranium (two per jar) were buried in the soil vertically (the sharp end down), and equidistant from the centre of the jar and its wall. After 12-months incubation, soil samples were removed for molecular characterization.

DNA extraction and purification

For F-RISA, 0.5 g aliquots of soil sample were taken from each microcosm vessel in triplicate and DNA was extracted using an Ultra-Clean Soil DNA isolation kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA was further purified by using a High Pure PCR product clean-up kit (Roche, Mannheim, Germany) according to the manufacturer's instructions except for the application of elution buffer. The DNAs were eluted in sterile Milli-Q water as the elution buffer supplied may interfere with the PCR and sequencing processes.

F-RISA PCR amplification

The fungal intergenic region containing the two ITS and the 5.8S rRNA gene (ITS1-5.8S-ITS2) was amplified using primer sets ITS1f (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') according to Gleeson *et al*. (2005). PCR primers were synthesized by the Oligonucleotide Synthesis Service, School of Life Sciences, University of Dundee. Negative controls were included in each reaction to ensure no contamination from experimental materials.

F-RISA procedure

To facilitate treatment comparisons within single gels, all PCR products from each treatment were pooled prior to analysis. Electrophoresis was performed with a Bio-Rad Protean II Xi Cell (Bio-Rad, Hercules, CA, USA) and 5% nondenaturing polyacrylamide gels were cast for F-RISA. 20 µl (approximately 400 ng) of each PCR product was mixed with 2 × gel loading dye (Bio-Rad) and electrophoresed at 60 V for 12 h at room temperature with 1 × tris acetate EDTA (TAE) buffer along with standard marker Step Ladder, 50 bp (Sigma, St Louis, MO, USA). Gels were stained with SYBR Green I (Sigma-Aldrich) for 30 min, and gel images were obtained using a ProXPRESS 2D Proteomic Imaging System (PerkinElmer, Munich, Germany).

F-RISA band DNA recovery and sequencing

Individual bands of interest were excised from polyacrylamide gels with a sterile surgical blade under UV. DNA was extracted using the modified crush and soak method (Maxam and Gilbert 1977; Hong *et al*. 2010). Each band was re-amplified using the original primer set as described previously and the PCR products were purified for the next steps using a High Pure PCR product clean-up kit. The re-amplified PCR products were cloned into the pDrive Cloning Vector (Qiagen, Hilden, Germany) according to the manufacturer's protocol and sequenced at the Sequencing Service, School of Life Sciences, University of Dundee (http://www.dnaseq.co.uk). The DNA sequences obtained in this study were submitted to the NCBI database with accession numbers from KM359786 to KM359798.

Results

The results of uranium measurements in the microcosms showed that the samples of metallic DU underwent corrosion and released mobile uranium species into their environment. This process was greatly influenced by the presence of a plant in the microcosms. Uranium concentration in the soil without a plant (3.12 ± 0.40 mg g^{-1} DW soil) was significantly higher than that in the microcosms with mycorrhizal (1.18 ± 0.14 mg g^{-1} DW soil) and non-mycorrhizal (0.80 ± 0.09 mg g^{-1} DW soil) plants. Specific accumulation of uranium in pine roots (3.42–7.26 mg g^{-1} DW biomass) and shoots (0.20–0.24 mg g^{-1} DW biomass) did not differ significantly for mycorrhizal and non-mycorrhizal plants. The data on uranium accumulation in the roots of non-mycorrhizal pines showed great variability. The uranium content within the microcosms in HNO_3-, acetic acid-, and water-soluble fractions demonstrated the same tendency as for uranium concentration per g of soil: the plant-free microcosms had a significantly higher uranium content in all fractions (Table 2). For the different microcosm variants, it was found that the order of the uranium content in soil per jar was plant-free > mycorrhizal pine > non-mycorrhizal pine with a ratio 3.9:1.5:1 for the HNO_3-fraction, 6.8:2.1:1 for the acetic acid fraction, and 3.8:1.2:1 for the water-soluble fraction. It was also found that uranium uptake by whole pine seedlings in the

mesocosms consisted of only approximately 0.1% and 0.4% of the HNO_3-fraction of uranium in soil for mycorrhizal and non-mycorrhizal pines (Table 2).

The F-RISA experiments demonstrated significant shifts in fungal communities inhabiting the microcosm soils in response to the DU contamination as well as to the introduction of mycorrhizal or non-mycorrhizal pines (Fig. 3). Distinct F-RISA target-bands of interest were identified for further manipulation and represented: (1) control MO band for the uncontaminated microcosm with the introduced *R. rubescens* B32o as a mycobiont of a pine seedling; (2) MU band for the DU contaminated microcosm with an ectomycorrhizal association; (3) NU band for the DU contaminated microcosm with non-mycorrhizal pines; and (4) OU band for DU contaminated pine-free microcosms. When comparing samples taken from uranium-contaminated (M/U) and uncontaminated (M/O) microcosms with the ectomycorrhizal association of Scots pine with *R. rubescens*, the MO1 band was clearly expressed in the gel in the M/O microcosm and barely manifest itself in the M/U variant (Fig. 3) whereas the MU1 band was present only in the M/U microcosm and absent in all other microcosm variants. For microcosms with non-mycorrhizal pines, the NU1 band drew attention, which appeared in the N/U microcosm and was absent in the uncontaminated microcosm (N/O) (Fig. 3). In the microcosms without pines (O/U and O/O), the OU12 band was chosen for further examination as this was clearly represented only in the O/U microcosm with soil contaminated with uranium. Subsequent cloning and sequencing of the target bands revealed that the ITS amplicons, co-migrated within the MU1 band and appearing only in the uranium-contaminated microcosms with ectomycorrhiza (M/U), were related to ectomycorrhizal fungi of the genus *Scleroderma* (Order Boletales, Phylum Basidiomycota) (Table 3). The nucleotide clone sequences obtained from the MO1 band, which appeared intensely in the control unpolluted M/O microcosm variant with mycorrhizal pine trees infected with *R. rubescens* (Order Boletales, Phylum Basidiomycota), were almost identical (1 bp difference) to the ITS sequence for the pure culture of this fungus that we used for pine mycorrhization (Table 3). This confirmed the successful

development of the ectomycorrhizal association introduced into the microcosms in the absence of depleted uranium pollution. The clones with an uncertain taxonomic affinity were recovered from the target band NU1, when DU contamination was combined with non-mycorrhizal pines in the microcosm (N/U) (Table 3). These could not be taxonomically resolved due to the lack of adequate information in the GenBank database. The closest matches to the NU1 clones were unknown fungal clones and uncultured clones related to the Zoopagomycotina subdivision (e.g. *Kuzuhaea moniliformis* and *Piptocephalis corymbifera*) with very low overlap (17%). In the case of microcosms without pines, it was found that the clones from the target band OU12, characteristic only for uranium-contaminated plant-free soil, were related to the *Mortierella* genus (Order Mortierellales, Subphylum Mortierellomycotina: Phylum Mucoromycota) (Table 3).

Discussion

One of the most serious environmental concerns is pollution by radionuclides, particularly uranium originating from the extraction and processing of uranium ores, the nuclear power industry, and anthropogenic disasters (e.g. the Chernobyl accident in 1986 and Fukushima-1 accident in 2011), as well as the use of depleted uranium for military purposes (Zhdanova *et al*. 2000; Salbu *et al*. 2005). Depleted uranium has no natural analogues and the environmental consequences of its introduction into the soil and food webs are poorly understood (Bleise *et al*. 2003). The effects of anthropogenic pollution by metallic depleted uranium on fungal communities in the soil and mycorrhizosphere have not been studied before. Introduction of thermodynamically unstable metallic uranium into the terrestrial environment must obviously affect the fungal communities that play an important role in element transformations and cycling. Studies on bacterial and archaean communities in uranium-contaminated soils using modern and classical microbiological methods have significantly outperformed those for fungal communities (Selenska-Pobell *et al*. 2001; Islam *et al*. 2011; Mondani *et al*. 2011; Kenarova *et al*. 2014;

Radeva *et al*. 2014). Following the development of culture-independent methods to characterize the diversity of bacterial communities, molecular biological techniques have increasingly been used to understand communities of fungi, primarily those that inhabit the soil and the rhizosphere (Buscot *et al*. 2000; Hodge 2000; Daniell *et al*. 2001; Burke *et al*. 2005; Jasrotia *et al*. 2014).

Despite the bias associated with PCR-based methodologies, the F-RISA technique provides one of the easiest and quickest fingerprinting means to compare the structure of fungal communities and to evaluate changes (Ranjard *et al*. 2001; Gleeson *et al*. 2005; Hong *et al*. 2010). Each band corresponds to one fungal species, also known as an operational taxonomic unit (OTU), and the number of bands is indicative of genetic diversity in the sample: the relative abundance of fungal species can be estimated by measuring the intensity of bands on the gel.

Our F-RISA results demonstrated that, under the selected experimental conditions, fungi responded to the presence of metallic depleted uranium by a shift in community composition, where the presence of a pine seedling and its mycorrhizal status had a significant effect on these processes (Fig. 4). The appearance of some bands is considered to reflect the response of the indigenous fungal community to the introduction of pine seedlings and, for mycorrhizal pines, with *R. rubescens* B32o, in the presence of DU. The ITS fragments from the band MU1 that appeared in DU-contaminated soil of the microcosms with mycorrhizal pines were related to ectomycorrhizal fungi of the genus *Scleroderma*, a well-known primary colonizer of metal-polluted terrestrial environments such as coal spoils and mining wastes (Jeffries 1999). The abundance of *R. rubescens* B32o, which was introduced as the ectomycorrhizal association with pine, decreased possibly being outcompeted by the indigenous *Scleroderma* under conditions of DU pollution.

The taxonomic affinity of the ITS fragments from the band that appeared in the non-mycorrhizal pines combined with DU microcosm was difficult to ascertain due to the lack of adequate information in the GenBank database and only suggested some possible relation to the

Zoopagomycotina. The results also revealed a rise of *Mortierella* sp. in the DU-polluted soil with plant-free microcosms (Fig. 4). Concurrently, the soil of such a microcosm variant contained the highest amount of uranium leached from the DU samples which was at least 3-4 times higher than the soil of pine containing microcosms in all chemical fractions, including the bioavailable water- and acetic acid soluble uranium species (Fig. 4). This could be explained by the presence of the pine plant resulting in uranium bioaccumulation within the plant biomass as well as in the difference in the water retention among the different microcosm variants, e.g. plant transpiration decreasing water in the soil and reducing uranium migration.

Nearly 100 taxonomically accepted species belong to the genus *Mortierella* are filamentous fungi commonly found in soil as saprotrophs. This genus has been suggested to be treated in its own subphylum Mortierellomycotina and is related to Mucoromycotina and Glomeromycotina (Yadav *et al*. 2015; Spatafora *et al*. 2016). Molecular phylogenetic analyses revealed considerable diversity within the Mortierellomycotina (Spatafora *et al*. 2016). Environmental sampling supports a great diversity of taxa associated with soils, rhizosphere, and plant roots (Spatafora *et al*. 2016). Soil *Mortierella* spp. are reported to exhibit resistance to toxic metals such as Cd, Hg, and Zn (Krantz-Rülcker *et al*. 1996) and capable of cadmium bioaccumulation (Ahmad *et al*. 2011) and cobalt sorption (Pal *et al*. 2006), although such properties are widely found in other fungi (Gadd 2007). It was also found that *Mortierella* and *Mucor* were the most abundant genera in As- and Hg-polluted soils of the Pestarena gold mine complex in Italy, irrespective of contamination levels (Crognale *et al*. 2017). It was also observed that there was a great abundance of *Mortierella* spp. in soil severely contaminated with radionuclides in the first few months after the Chernobyl disaster in Ukraine (Prof. Nelli Zhdanova, personal communication). It should be noted that phylogenetic analysis of *Mortierella* sp. clone OU12-14 (KM359797) in our experimental microcosm study showed the closest match to the ITS sequences of the *Mortierella* sp. DU17 (KM113754) strain isolated from depleted uranium deteriorated under natural conditions in grassland soil in a DU testing

range (Kirkcudbright, Scotland, UK) (Fomina and Gadd, unpublished). In conclusion, our F-RISA study has demonstrated that soil contamination by depleted uranium can cause a pronounced shift in the composition of a soil fungal community, which is affected by the presence of a plant and its mycorrhizal status, and may therefore influence the biogeochemical fate of DU.

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Legends to Figures

Fig 1. The samples of depleted uranium used in this research.

Fig. 2. Soil microcosm design. (A) diagrammatic representation (see Methods and materials for description); (B) a typical control DU-free microcosm with a mycorrhizal pine seedling (M/O) (image taken at the time of watering, lid not present).

Fig. 3. F-RISA: gel image of fungal community composition in microcosm soil samples. Lanes M: marker; lane M/U: microcosm variant with mycorrhizal pine and DU-polluted soil; lane M/O: microcosm with mycorrhizal pine and non-polluted reference soil; lane N/U: microcosm with non-mycorrhizal pine and DU-polluted soil; lane N/O: microcosm with non-mycorrhizal pine and non-polluted soil; lane O/U: pine-free microcosm with DU-polluted soil; lane O/O: pine-free microcosm with non-polluted soil. White arrows indicate the bands of interest. A typical gel is shown (n=4).

Fig. 4. Diagram summarizing the consequences of DU contamination of soil for fungal communities in relation to the presence or absence of mycorrhizal and non-mycorrhizal plants: plant absence leads to the highest total uranium accumulation (U_{HNO3}) in the soil of the microcosm especially for bioavailable uranium species (water- and acetic acid-soluble fractions: U_{H2O} and U_{AA}).